

# Floral isolation between *Aquilegia formosa* and *Aquilegia pubescens*

Michelle Fulton and Scott A. Hodges\*

Department of Ecology, Evolution and Marine Biology, University of California, Santa Barbara, CA 93106, USA  
and The White Mountain Research Station, 3000 East Line Street, Bishop, CA 93514, USA

The acquisition of floral nectar spurs is correlated with increased species diversity across multiple clades. We tested whether variation in nectar spurs influences reproductive isolation and, thus, can potentially promote species diversity using two species of *Aquilegia*, *Aquilegia formosa* and *Aquilegia pubescens*, which form narrow hybrid zones. Floral visitors strongly discriminated between the two species both in natural populations and at mixed-species arrays of individual flowers. Bees and hummingbirds visited flowers of *A. formosa* at a much greater rate than flowers of *A. pubescens*. Hawkmoths, however, nearly exclusively visited flowers of *A. pubescens*. We found that altering the orientation of *A. pubescens* flowers from upright to pendent, like the flowers of *A. formosa*, reduced hawkmoth visitation by an order of magnitude. In contrast, shortening the length of the nectar spurs of *A. pubescens* flowers to a length similar to *A. formosa* flowers did not affect hawkmoth visitation. However, pollen removal was significantly reduced in flowers with shortened nectar spurs. These data indicate that floral traits promote floral isolation between these species and that specific floral traits affect floral isolation via ethological isolation while others affect floral isolation via mechanical isolation.

**Keywords:** reproductive isolation; speciation; adaptive radiation; species diversification

## 1. INTRODUCTION

Adaptive radiations can occur either after species invade a new habitat such as an oceanic island or after the evolution of a new character that allows the species to use resources in a novel manner (Simpson 1953). Such novel characters are often termed 'key innovations' and in some instances can lead to an increase in the species diversity of clades that possess them. Recently, many workers have focused on methods for statistically evaluating suspected key innovations. These methods either test for a correlation between the inferred time that a trait evolves and the inferred time of a change in diversification (e.g. Sanderson & Donoghue 1994; Wollenberg *et al.* 1996) or test for a correlation across multiple pairs of sister taxa between the evolution of a trait and increased species diversity (e.g. Guyer & Slowinski 1993; Slowinski & Guyer 1993; Barraclough *et al.* 1995; Goudet 1999). Despite these advances, a significant positive correlation from these tests, while compelling, could be spurious if the suspected key innovation is correlated with another factor that actually causes diversification.

The species diversity of a clade depends on its rates of species formation and extinction and, therefore, one way to test a key innovation hypothesis is to determine whether a trait can affect either of these processes. The evolution of reproductive isolation is central to most concepts of speciation (Dobzhansky 1937; Mayr 1942; Grant 1963) and, therefore, in order to understand whether a proposed key innovation can potentially

influence species diversification, it is important to determine whether a character can promote reproductive isolation. Many factors, including mating patterns, gamete competition, genetic compatibility and ecology, can influence the degree of reproductive isolation between species (Harrison 1990; Coyne & Orr 1998; Schluter 1998). If a character evolves which significantly increases the likelihood or degree of reproductive isolation among the taxa that possess it the character will promote species diversity and can be considered a key innovation.

Among flowering plants, floral isolation has long been suggested as a major avenue for achieving reproductive isolation and influencing species diversity (Grant 1949). Floral isolation may act through either differential pollination due to pollinator behaviour (ethological isolation) or through differential pollen transfer (mechanical isolation) (Grant 1949). The evolution of floral nectar spurs has been proposed as an example of a key innovation through its effects on floral isolation. Nectar spurs are tubular outgrowths of floral parts with nectar produced at their tips. Across multiple independent lineages, the evolution of nectar spurs is highly correlated with significant increases in species numbers (Hodges & Arnold 1995; Hodges 1997*a,b*). Nectar spurs could promote floral isolation because changes in morphology and colour may reduce or enhance visitation or the transfer of pollen by different types of pollinators. Species with spurs may therefore achieve a greater degree of reproductive isolation than species lacking spurs (Hodges & Arnold 1995; Hodges 1997*a,b*).

The genus *Aquilegia* presents a prime example of the correlation between nectar spurs and species diversity.

\*Author for correspondence (hodges@lifesci.ucsb.edu).

Molecular phylogenetic evidence suggests that *Aquilegia* has undergone a recent and rapid radiation of taxa after the evolution of floral nectar spurs (Hodges & Arnold 1994a, 1995). However, whether floral characters can influence reproductive isolation in *Aquilegia* has been controversial. Grant (1952) used two species of *Aquilegia* (*A. formosa* and *A. pubescens*) as the first example of how floral isolation could operate in nature. He observed hummingbirds foraging at the scarlet-red and yellow, pendent, short-spurred flowers of *A. formosa* and hawkmoths foraging at the white or pale yellow, upright, long-spurred flowers of *A. pubescens*. Because these plant–pollinator associations were observed even in hybrid zones between the species, Grant (1952) suggested that the behaviour of the pollinators would largely keep their gene pools separate. However, Chase & Raven (1975) reported observing both hummingbirds and hawkmoths visiting both *A. formosa* and *A. pubescens* and concluded that there was no floral isolation. Grant (1976) questioned the conclusions of Chase & Raven (1975) partially because they lacked data on the frequency of floral visitors to both species simultaneously.

Although species that form hybrid zones have not fully attained reproductive isolation they offer the unique opportunity of determining whether specific characters contribute to reproductive isolation (Harrison 1990). Hodges & Arnold (1994b) scored floral and molecular characters across hybrid zones between *A. formosa* and *A. pubescens* and concluded from the shapes of the clines that selection acts against the introgression of most floral characters across the hybrid zone. However, cline shapes only indicate that selection influences a particular trait, not whether selection acts directly on the trait or on genes linked to it. To understand whether floral isolation could have influenced the rapid species diversification in *Aquilegia*, we estimated floral isolation across a narrow hybrid zone between *A. formosa* and *A. pubescens*. We observed floral visitors in natural populations of both species simultaneously, tested the preferences of pollinators using arrays of flowers and tested how specific floral characters affect isolation between these species.

## 2. MATERIAL AND METHODS

The study sites were in the southern Sierra Nevada mountains (Inyo County, CA) following portions of the Bishop Creek drainage: an *A. formosa* site along Bishop Creek (2365 m), an *A. formosa* site (3304 m) and an *A. pubescens* site (3267 m) near Lamarck Lakes (these two sites are separated by ca. 400 m), a hybrid population, an *A. pubescens* site near Loch Leven (3276 m) and *A. pubescens* sites near Lamarck Col (3900 m) and Piute Pass (3560 m).

Simultaneous 1 h-long observations of floral visitors were made every other hour from 08.00 until dark (ca. 20.30) on 4–8 August 1997 at all seven sites. At each site a patch of flowers was observed and the total number of open flowers in the patch was recorded during each observation period. The total number of flowers visited in the patch and the time of the visit were recorded for each floral visitor. In addition, on a subset of visits by hummingbirds and hawkmoths, we recorded the number of spurs on each flower that were probed.

The preferences of the pollinator classes for one or the other species of *Aquilegia* were tested using arrays of flowers. Each

array consisted of nine newly opened flowers of each species which were placed in florist's water pics (plastic vials for holding flowers) arranged alternately in a hexagonal pattern at 15-cm intervals. The arrays were placed near patches of *A. formosa* between ca. 08.00 and 12.00 and near patches of *A. pubescens* at 19.30–20.00 until dark. A total of 16 arrays were observed on 12–15 August 1997 near *A. formosa* and a total of eight arrays were observed on 11–13 August 1997 near *A. pubescens*. Flowers were replaced daily if they began to age or wilt. For each floral visitor its type and pattern of visits was recorded. These experiments were conducted at the Lamarck Lake sites where both species occur.

To test whether specific floral characters influenced the pollinator visitation patterns, we constructed arrays of flowers from a single species (*A. pubescens*) and then manipulated half the flowers to mimic a character state of *A. formosa*. Two experiments were conducted: (i) the pedicels of half the flowers were tied to small stakes to make the flowers pendent (six arrays observed), and (ii) half the flowers had their nectar spurs shortened to ca. 2 cm in length (two arrays observed). To shorten the nectar spurs we first squeezed any nectar up the nectar spur and then tied the spur off with thread and trimmed off the remaining nectar spur below the point where it was tied. The arrays were set out between 19.30 and 20.30 in the *A. pubescens* site at Lamarck Lakes between 12 and 14 August 1997 and hawkmoth visits were recorded as described above.

To test whether mechanical isolation was acting between normal *A. pubescens* flowers and those with shortened nectar spurs, we measured the number of pollen grains remaining in the anthers after hawkmoth visitation. During the morning prior to presenting the arrays to hawkmoths, we removed opened anthers from which pollen may have previously been removed. During the day new anthers opened and were available for pollen removal during the observation period. After visitation was complete, we collected the open anthers in micro-centrifuge tubes and allowed them to dry completely. We counted the number of pollen grains remaining using an Elzone particle counter using the methods of Mazer & Schick (1991).

## 3. RESULTS

The mean number of visits to flowers per hour by each class of floral visitor (bees, flies, hummingbirds and hawkmoths) was calculated for each site and observation period. The total number of flower visits by each pollinator class was divided by the total number of flowers in the observation patch and divided by the length of the observation period. Most observation periods were 1 h in length but some were shorter due to poor weather or because it became too dark to observe visitation. Bees and flies were observed to visit flowers to collect pollen. Hummingbirds and hawkmoths foraged for nectar and generally probed multiple spurs per flower visit. Each probe of a spur is similar to a single flower visit and both hummingbirds ( $n=12$  and s.e.=0.50) and hawkmoths ( $n=30$  and s.e.=0.52) probed an average of 3.3 spurs per flower visit. Thus, we multiplied the number of flowers visited by hawkmoths and hummingbirds by 3.3.

ANOVA was used to test the effects of plant species, class of visitor and observation time on the mean visits per flower per hour. The data from the hybrid site were excluded from this analysis because only a single site was observed. A nearly significant ( $p<0.10$ ) effect due to

Table 1. ANOVA of the effects of species (*A. formosa* or *A. pubescens*), type of visitor (fly, bee, hummingbird or hawkmoth) and time of day on visits per flower per hour

source of variation	d.f.	SS	MS	F	p
species	1	0.18	0.18	3.51	0.0620
visitor type	3	0.11	0.04	0.54	n.s.
time	6	0.31	0.05	1.02	n.s.
species × visitor type	3	1.32	0.44	8.77	<0.0001
species × time	6	1.44	0.24	4.79	<0.0001
visitor type × time	18	3.78	0.21	4.20	<0.0001
species × visitor type × time	18	2.51	0.14	2.78	<0.0001
residual	503	25.17	0.05	—	—

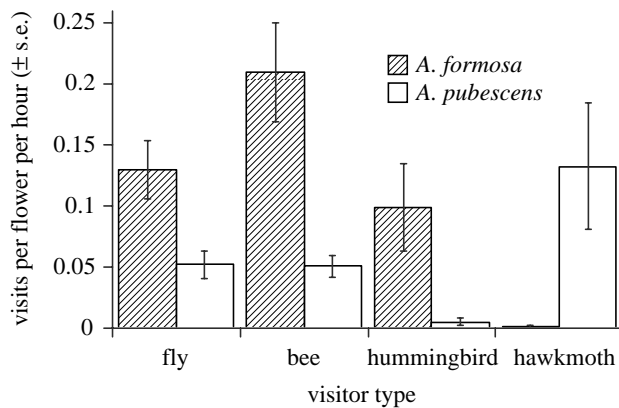


Figure 1. Mean visits per flower per hour by different classes of pollinators to *A. formosa* and *A. pubescens*. Plants at multiple sites were observed simultaneously for 1 h every other hour from 08.00 to dark over a five-day period.

plant species was found but no significant effects were found for visitor class or time of day (table 1). *Aquilegia formosa* received more flower visits overall than *A. pubescens*. There was a highly significant interaction between plant species and visitor class (table 1) due to high hummingbird visitation to *A. formosa* but not to *A. pubescens* and high hawkmoth visitation to *A. pubescens* but not to *A. formosa* (figure 1). Significant interaction terms were also found for plant species × time, visitor class × time and the three-way interaction (table 1). These differences appeared to be mainly due to high hummingbird visitation to *A. formosa* during the morning and very high hawkmoth visitation to *A. pubescens* at the 20.00 observation period (data not shown).

For the *A. formosa* and *A. pubescens* sites separated by only 400 m, the differences in visitation patterns between pollinator classes were similar to those found across all sites. At these two sites, *A. formosa* received averages of 0.06, 0.28, 0.18 and 0.003 visits per flower per hour and *A. pubescens* received averages of 0.04, 0.06, 0.006 and 0.15 visits per flower per hour by flies, bees, hummingbirds and hawkmoths, respectively. The single hybrid population observed contained mostly individuals whose flowers resembled *A. pubescens*. This patch was predominantly visited by hawkmoths (0.29 visits per flower per hour), while hummingbirds, bees and flies had visitation rates of 0.04, 0.30 and 0.02 visits per flower per hour, respectively.

Table 2. Pollinator preferences for flowers of *A. formosa* and *A. pubescens* arranged alternately in a hexagonal array

(Each pollinator class was tested against an expected 1:1 visitation to the two species.)

visits to	observed number of visits		
	hummingbirds	hawkmoths	bees
<i>A. formosa</i>	81	0	85
<i>A. pubescens</i>	9	115	19
$\chi^2$	57.6	115	41.88
p	<0.0001	<0.0001	<0.0001

Table 3. Visitations by hawkmoths (*Hyles lineata*) to upright and pendent (manipulated) flowers of *A. pubescens* arranged alternately in a hexagonal array

visits to	number of visits observed
upright	59
pendent	5
$\chi^2$	45.45
p	<0.0001

Table 4. Visitations by hawkmoths (*H. lineata*) to flowers of *A. pubescens* with long or short (manipulated) nectar spurs arranged alternately in a hexagonal array

visits to	number of visits observed
long	17
short	19
$\chi^2$	0.11
p	n.s.

In the mixed-species arrays, each class of floral visitor strongly discriminated between *A. formosa* and *A. pubescens* (table 2). Just as in the natural stands, hummingbirds and bees showed strong preferences for *A. formosa*, while hawkmoths showed complete fidelity to *A. pubescens*. In each of the four instances when hummingbirds were observed to visit flowers of both species they always visited more flowers of *A. formosa*. Of the 39 bee visits observed, 34 were to one species only, nine to *A. pubescens* and 25 to *A. formosa*.

In arrays of *A. pubescens* with half the flowers altered to have pendent flowers like *A. formosa*, hawkmoths visited upright flowers more than ten times as often as pendent flowers (table 3). In contrast, when half the flowers were altered to have short spurs like *A. formosa*, hawkmoths showed no preference between long- and short-spurred flowers (table 4). However, pollen removal was significantly reduced in short-spurred flowers as higher numbers of pollen grains remained in their anthers after visitation ( $t = 2.52$ , d.f. = 14 and  $p < 0.025$ ; figure 2).

#### 4. DISCUSSION

Reproductive isolation between *A. formosa* and *A. pubescens* is clearly influenced by floral isolation due to both

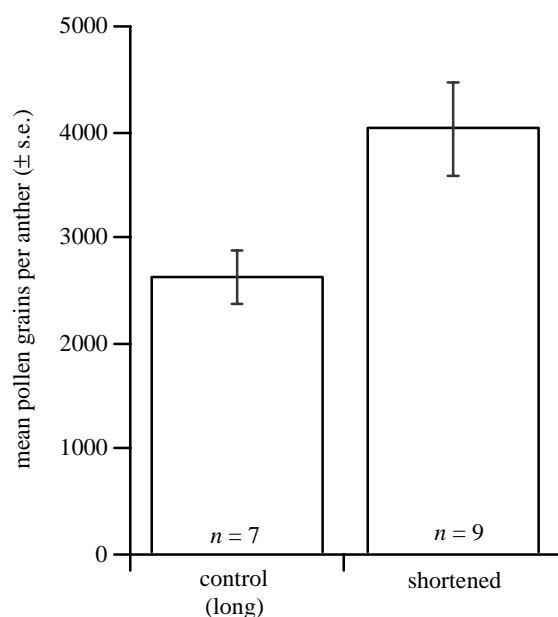


Figure 2. Mean number of pollen grains remaining in the anthers of flowers of *A. pubescens* after hawkmoth visitation. Prior to hawkmoth visitation, nectar spurs were either shortened to a length similar to the nectar spurs of *A. formosa* or left unmanipulated (long).

pollinator behaviour (ethological isolation) and mechanical isolation. All classes of floral visitors had significantly different visitation patterns to the two species (table 1 and figure 1), even when the species were in close proximity (400 m). For the visitor classes which probably affect pollination, the same visitation patterns were observed in mixed arrays of flowers of the two species (table 2). In particular, as described by Grant (1952, 1976), in natural populations we observed high visitation to *A. formosa* by hummingbirds but not hawkmoths and high visitation to *A. pubescens* by hawkmoths but not hummingbirds. Thus, these pollinators will cause assortative mating due to floral characters even when the species co-occur. Furthermore, hummingbirds and hawkmoths are likely to be highly effective pollinators for the species that they visit because pollen is transferred onto their bodies as they probe the spurs and both male and female phase flowers are routinely visited (S. A. Hodges, personal observation).

Bees and flies made large numbers of visits to both *A. formosa* and *A. pubescens* (figure 1) and, therefore, it could be inferred that this behaviour weakens any floral isolation brought about by differential hummingbird and hawkmoth visitation. However, in this system flies are unlikely to affect pollination. Most fly visits were from syrphid flies that hovered in front of a flower and then alighted on a single anther without contacting receptive stigmas. Furthermore, although bees as a class may visit both species, the fidelity of individual bees to one or the other species will result in assortative mating. Out of the bees observed at our mixed species arrays, 87% visited flowers of only one species. In a similar experiment, Anderson & Schafer (1933) planted species of *Aquilegia* from the *vulgaris*, *alpina*, *canadensis* and *caerulea* groups together (bee, bee, hummingbird and hawkmoth pollination syndromes, respectively, as described by Grant

(1952)) but found no hybrid seeds produced on a double recessive *Aquilegia vulgaris* plant. Anderson & Schafer (1933) attributed the lack of hybrids to individual bee fidelity. In addition, bees may not severely impact floral isolation if they are poor pollen vectors. Bees visited *Aquilegia* flowers to collect pollen and therefore may avoid female-phase flowers. For example, Miller (1981) reported that pollen-collecting bees generally avoided emasculated flowers of *Aquilegia caerulea* while nectar-foraging hawkmoths did not.

Although it has long been held that selection by pollinators is a major cause of plant speciation (Grant 1949, 1971; Stebbins 1970), the importance of floral specialization and isolation has recently been questioned (e.g. Waser *et al.* 1996; Waser 1998). These authors argued that most animals visit many plant species and that most plant species receive visits from many types of animals. This apparent lack of fidelity could severely weaken floral isolation. However, as we have shown here, despite four distinct groups of animals visiting *A. formosa* and *A. pubescens*, floral isolation still occurs. Once the frequency of visits, the fidelity of individual pollinators and the effectiveness of pollinators were considered, we found strong assortative mating due to floral differences.

Floral isolation is likely to be only one of several factors that together provide sufficient reproductive isolation to keep *A. formosa* and *A. pubescens* as distinct taxa (Grant 1992). Pollen competition and seed abortion may limit hybrid seed production (Carney *et al.* 1996; Emms *et al.* 1996) and selection may act against the establishment of hybrid plants. For instance, selection against hybrids probably occurs during seedling establishment due to the different soil requirements of *A. formosa* and *A. pubescens* (Grant 1952; Chase & Raven 1975; Hodges & Arnold 1994b). Finally, pollinators may cause selection against plants with hybrid morphologies (e.g. table 2 and figure 2). In the future it will be important to determine the relative contribution of each life-history stage to the overall reproductive isolation between these species.

Here we have shown that flower orientation affects floral isolation ethologically (table 3) while spur length influences floral isolation mechanically (table 4). It is likely that other floral characters also influence reproductive isolation between *A. formosa* and *A. pubescens*. Making flowers of *A. pubescens* pendent severely reduced hawkmoth visitation, but visitation still occurred. However, hawkmoths completely avoided *A. formosa* flowers in all of our observations (table 2). Thus, some other character, such as flower colour, probably interacts with flower orientation to prevent hawkmoth visitation to *A. formosa*. *Aquilegia formosa* flowers reflect far less light than *A. pubescens* flowers (S. A. Hodges, unpublished data) making them difficult to observe at dusk when hawkmoths are active. Other floral characters that may contribute to ethological isolation are floral odour and nectar production. Additional scenarios can be constructed for these other floral characters and, as we have shown here, direct tests of these hypotheses are possible and should be conducted to understand fully how floral isolation operates between these two species.

Few other studies have directly measured the extent of floral isolation between species. Two species of *Silene*

(*Silene dioica* and *Silene latifolia*) are intercompatible yet remain largely distinct. Using pollinator observations, Goulson & Jerrim (1997) found that floral visitors had strong preferences for different species, with bumblebees visiting *S. dioica* and moths visiting *S. latifolia*. These behaviours translated into assortative mating as judged by pollen analogues (i.e. fluorescent dyes), which were transferred largely within species. However, Goulson & Jerrim (1997) concluded that floral isolation is weak between these species because allozyme data showed that the species and their hybrids were not well differentiated. However, none of the allozymes in their study showed fixed allelic differences between the species in allopatry. Thus, the retention of an ancestral polymorphism or stabilizing selection could also explain similar allele frequencies between sister taxa (e.g. Karl & Avise 1992). Differential visitation between species has also been found in *Ipomopsis* (Campbell *et al.* 1997) and *Mimulus* (Sutherland & Vickery 1993).

Though floral isolation is presently operating between *A. formosa* and *A. pubescens*, it is not clear whether it has promoted speciation in *Aquilegia* or whether it evolved after speciation occurred due to other factors. If floral isolation directly promotes speciation and is largely responsible for the rapid radiation of the genus, then we would expect shifts in pollination syndrome to be correlated with speciation events. Thus, a species-level phylogeny would provide the opportunity of testing this hypothesis. Inferred shifts in pollination syndrome across the phylogeny should occur significantly more often than random. For example, Johnson *et al.* (1998) found that shifts in pollination syndrome were highly correlated with speciation in *Disa*, an orchid genus where most species possess floral spurs. Alternative hypotheses such as the importance of shifts in habitat usage could also be tested (e.g. Barraclough *et al.* 1998). Unfortunately, speciation has apparently occurred so rapidly in *Aquilegia* that the individual gene regions sampled so far do not harbour sufficient nucleotide variation to reconstruct a reliable phylogeny of the genus (Hodges & Arnold 1994a).

Floral isolation can also increase species diversity by reducing the rate of extinction. Hybridization and introgression can cause extinction by merging previously distinct taxa into a single species (Levin *et al.* 1996). Thus, taxa that lack floral specialization and isolation may be more likely to merge during secondary contact after allopatric divergence. We would therefore expect to find more frequent or extensive geographical overlap among related species possessing spurs than among species in their non-spurred sister taxa. Detailed phylogenetic tests of the process of speciation, as well as studies focused on how species diversity is maintained, are needed to understand how the evolution of nectar spurs may have promoted species diversity in *Aquilegia* and other groups of flowering plants.

The authors thank the members of the Interagency Research Team, J. Beck, K. Brown, J. Downing, C. Hancock, H. Johnson, J. Mangin, P. McGrath, A. Sada, S. Szewczak, D. Warren and P. Wundrow, as well as E. Henry, D. Plaister and J. Szewczak who helped make pollinator observations and collect samples, J. Damuth, S. Mazer, B. Larson, L. Chittka and two anonymous reviewers for helpful suggestions and S. Mazer for use of the Elzone particle counter. This study was funded by National

Foundation grant DEB-9726272 and a White Mountain Research Station Faculty Research grant to S.A.H.

## REFERENCES

- Anderson, E. & Schafer, B. 1933 Vicinism in *Aquilegia*. *Am. Nat.* **67**, 190–192.
- Barraclough, T. G., Harvey, P. H. & Nee, S. 1995 Sexual selection and taxonomic diversity in passerine birds. *Proc. R. Soc. Lond. B* **259**, 211–215.
- Barraclough, T. G., Vogler, A. P. & Harvey, P. H. 1998 Revealing the factors that promote speciation. *Phil. Trans. R. Soc. Lond. B* **353**, 241–249.
- Campbell, D. R., Waser, N. M. & Melendez-Ackerman, E. J. 1997 Analyzing pollinator-mediated selection in a plant hybrid zone: hummingbird visitation patterns on three spatial scales. *Am. Nat.* **149**, 295–315.
- Carney, S. E., Hodges, S. A. & Arnold, M. L. 1996 Effects of differential pollen-tube growth on hybridization in the Louisiana irises. *Evolution* **47**, 1432–1445.
- Chase, V. C. & Raven, P. H. 1975 Evolutionary and ecological relationships between *Aquilegia formosa* and *A. pubescens* (Ranunculaceae), two perennial plants. *Evolution* **29**, 474–486.
- Coyne, J. A. & Orr, H. A. 1998 The evolutionary genetics of speciation. *Phil. Trans. R. Soc. Lond. B* **353**, 287–305.
- Dobzhansky, T. 1937 *Genetics and the origin of species*. New York: Columbia University Press.
- Emms, S. K., Hodges, S. A. & Arnold, M. L. 1996 Pollen-tube competition, siring success and consistent asymmetric hybridization in the Louisiana irises. *Evolution* **50**, 2201–2206.
- Goudet, J. 1999 An improved procedure for testing the effects of key innovations on rate of speciation. *Am. Nat.* **153**, 549–555.
- Goulson, D. & Jerrim, K. 1997 Maintenance of the species boundary between *Silene dioica* and *S. latifolia* (red and white campion). *Oikos* **79**, 115–126.
- Grant, V. 1949 Pollination systems as isolating mechanisms in angiosperms. *Evolution* **3**, 82–97.
- Grant, V. 1952 Isolation and hybridization between *Aquilegia formosa* and *A. pubescens*. *Aliso* **2**, 341–360.
- Grant, V. 1963 *The origin of adaptations*. New York: Columbia University Press.
- Grant, V. 1971 *Plant speciation*. New York: Columbia University Press.
- Grant, V. 1976 Isolation between *Aquilegia formosa* and *A. pubescens*: a reply and reconsideration. *Evolution* **30**, 625–628.
- Grant, V. 1992 Floral isolation between ornithophilous and sphingophilous species of *Ipomopsis* and *Aquilegia*. *Proc. Natl Acad. Sci. USA* **89**, 11 828–11 831.
- Guyer, C. & Slowinski, J. B. 1993 Adaptive radiation and the topology of large phylogenies. *Evolution* **47**, 253–263.
- Harrison, R. 1990 Hybrid zones: windows on evolutionary process. In *Oxford surveys in evolutionary biology*, vol. 7 (ed. D. Futuyama & J. Antonovics), pp. 69–128. Oxford University Press.
- Hodges, S. A. 1997a Floral nectar spurs and diversification. *Int. J. Plant Sci.* **158**, S81–S88.
- Hodges, S. A. 1997b A rapid adaptive radiation via a key innovation in *Aquilegia*. In *Molecular evolution and adaptive radiations* (ed. T. Givinish & K. Sytsma), pp. 391–405. Cambridge University Press.
- Hodges, S. A. & Arnold, M. L. 1994a Columbinines: a geographically wide-spread species flock. *Proc. Natl Acad. Sci. USA* **91**, 5129–5132.
- Hodges, S. A. & Arnold, M. L. 1994b Floral and ecological isolation between *Aquilegia formosa* and *Aquilegia pubescens*. *Proc. Natl Acad. Sci. USA* **91**, 2493–2496.
- Hodges, S. A. & Arnold, M. L. 1995 Spurring plant diversification: are floral nectar spurs a key innovation? *Proc. R. Soc. Lond. B* **262**, 343–348.

- Johnson, S. D., Linder, H. P. & Steiner, K. E. 1998 Phylogeny and radiation of pollination systems in *Disa* (Orchidaceae). *Am. J. Bot.* **85**, 402–411.
- Karl, S. A. & Avise, J. C. 1992 Balancing selection at allozyme loci in oysters: implications from nuclear RFLPs. *Science* **256**, 100–102.
- Levin, D. A., Francisco-Ortega, J. & Jansen, R. K. 1996 Hybridization and the extinction of rare plant species. *Conserv. Biol.* **10**, 10–16.
- Mayr, E. 1942 *Systematics and the origin of species*. New York: Columbia University Press.
- Mazer, S. J. & Schick, C. T. 1991 Constancy of population parameters for life-history and floral traits in *Raphanus sativus* L. 2. Effects of planting density on phenotype and heritability estimates. *Evolution* **45**, 1888–1907.
- Miller, R. B. 1981 Hawkmoths and the geographic patterns of floral variation in *Aquilegia caerulea*. *Evolution* **35**, 763–774.
- Sanderson, M. J. & Donoghue, M. J. 1994 Shifts in diversification rate with the origin of angiosperms. *Science* **264**, 1590–1593.
- Schluter, D. 1998 Ecological causes of speciation. In *Endless forms: patterns and processes of speciation* (ed. D. Howard & S. Berlocher), pp. 114–129. Oxford University Press.
- Simpson, G. G. 1953 *The major features of evolution*. New York: Columbia University Press.
- Slowinski, J. B. & Guyer, C. 1993 Testing whether certain traits have caused amplified diversification: an improved method based on a model of random speciation and extinction. *Am. Nat.* **142**, 1019–1024.
- Stebbins, G. 1970 Adaptive radiation of reproductive characteristics in angiosperms. I. Pollination mechanisms. *A. Rev. Ecol. Syst.* **1**, 307–326.
- Sutherland, S. D. & Vickery Jr, R. K. 1993 On the relative importance of floral color shape and nectar rewards in attracting pollinators to *Mimulus*. *Great Basin Nat.* **53**, 107–117.
- Waser, N. M. 1998 Pollination, angiosperm speciation, and the nature of species boundaries. *Oikos* **82**, 198–201.
- Waser, N. M., Chittka, L., Price, M. V., Williams, N. M. & Ollerton, J. 1996 Generalization in pollination systems, and why it matters. *Ecology* **77**, 1043–1060.
- Wollenberg, K., Arnold, J. & Avise, J. C. 1996 Recognizing the forest for the trees: testing temporal patterns of cladogenesis using a null model of stochastic diversification. *Mol. Biol. Evol.* **13**, 833–849.